



Florida State University Animal Care and Use Committee

Guidelines On Euthanasia Using Carbon Dioxide

Euthanasia of animals must be carried out using an acceptable or acceptable with conditions method listed in the most recent edition of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals or must be scientifically justified and approved in advance by the FSU ACUC. The use of carbon dioxide (CO₂) inhalation is a method currently considered acceptable with conditions and is one of the most common methods of euthanasia used for small mammals and birds in research. It is the default method used by FSU Laboratory Animal Resources staff with animals identified for euthanasia by researchers.

Exposure to high concentrations of CO₂ has an initial rapid depressant and narcosis effect followed by death through asphyxiation. Unconsciousness is induced rapidly by exposing animals to a high CO₂ concentration (70% or more), however animals can experience pain and distress when exposed to sudden high concentrations of carbon dioxide. Carbon dioxide has the potential to cause distress in animals through three different mechanisms:

- pain due formation of carbonic acid when it contacts moisture on the respiratory and ocular membranes
- production of so-called air hunger and a feeling of breathlessness
- direct stimulation of ion channels within the amygdala associated with the fear response

Induction of loss of consciousness at concentrations < 80% may produce postmortem pulmonary and upper respiratory tract lesions. Because a gentle death that takes longer is preferable to a rapid, but more distressing death, gradual-fill CO₂ exposure is the only method approved for use at Florida State University. If an appropriate gradual displacement rate is used, animals will lose consciousness before CO₂ concentrations become painful.

Appropriate technique, equipment, and source of CO₂ must be used. Compressed CO₂ gas in cylinders is the only approved source because the inflow of gas to the euthanasia chamber can be regulated precisely. CO₂ generated by other means such as dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable.

Because the most common errors that have an impact on humane euthanasia when using CO₂ for euthanasia are: (1) overcrowding animals in the chamber, (2) using equipment or methods that cause the animals to be exposed to suboptimal concentrations, and (3) not assuring that animals are truly dead prior to disposal, the following standards must be adhered to when using CO₂ to euthanize animals:

- Only gradual fill (10-30% chamber volume/min) may be used. Flow rates for mouse and rat boxes have been calculated and are posted at all LAR carbon dioxide stations. This requires the use of a pressure regulator and a flow meter. No pre-filling of chambers is permitted.
- Euthanasia chambers must not be overcrowded. Minimum space requirements as listed in the "Guide" are recommended for all animals undergoing euthanasia with CO₂. There must be sufficient room in the chamber for normal postural movements for all the animals.
- It is recommended to euthanize animals in their home cages. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses to remove feces, urine and pheromones that can cause distress to the next group of animals. Placing live animals in with recently deceased animals is prohibited.
- To further reduce distress, euthanasia should be performed out of direct sight of other animals.
- Do not mix animals from different cages to avoid inducing stress and fighting. Never mix species in the same chamber when performing CO₂ euthanasia.
- The euthanasia chamber must be clear or have a clear window or lid for observation of the animals.
- Carbon dioxide is 50% heavier than air, therefore chambers should be designed so that as they fill with gas they can vent from the top, allowing air to exit at the top and be replaced by carbon dioxide. Incomplete filling of a chamber may permit tall or climbing animals to avoid exposure to an optimal concentration of gas, which can lead to prolonged distress to the animals.
- Since the effects of CO₂ are reversible, animals that are prematurely removed from the chamber prior to death can recover. Death must be confirmed by someone specifically trained to recognize cessation of vital signs in rodents. Death may be confirmed by physical examination or ensured by an adjunctive physical method. If an animal is not dead, CO₂ narcosis must be followed with another method of euthanasia. Animals may not be placed into a morgue freezer until after death has been confirmed. **Failure to ensure death of animals after euthanasia procedures is a reportable instance of non-compliance and violates PHS policy and the Animal Welfare Act.**
- Due to respiratory adaptations in immature animals, reptiles, amphibians, and some burrowing and diving species, consideration must be given in advance to the use of extended exposure times to high CO₂ concentrations following induction of unconsciousness, follow-up exposure to hypoxemia, or a secondary euthanasia method to ensure death.
- All individuals responsible for administering CO₂ euthanasia must be trained and qualified to perform euthanasia humanely. Training is available from Laboratory Animal Resources Staff.

See Also:

FSU ACUC Euthanasia Guidelines

Standard Operating Procedure, Euthanasia Of Rodents And Birds Using Carbon Dioxide

References:

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- Yavari P, McCulloch PF, Panneton WM. Trigeminally-mediated alteration of cardiorespiratory rhythms during nasal application of carbon dioxide in the rat. *J Auton Nerv Syst* 1996;**61**:195–200.